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IN VIVO METABOLISM OF α,α,β,β-TETRADEUTERO-*N*, *N*-DIMETHYLTRYPTAMINE IN RODENT BRAIN*†

STEVEN A. BARKER,‡ JOHN M. BEATON, SAMUEL T. CHRISTIAN, JOHN A. MONTI and PHILIP E. MORRIS

The Neurosciences Program and the Department of Psychiatry, University of Alabama in Birmingham, Birmingham, AL 35294, U.S.A.

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Abstract—The metabolism of α,α,β,β-tetradeutero-*N*,*N*-dimethyltryptamine (D_4DMT) in rat brain *in vivo* as a function of time and dose was examined. Quantification of D_4DMT and its respective deutero-metabolites was accomplished using gas chromatographic/mass spectrometric/selected ion monitoring/isotope dilution techniques. The results of this study indicate that D_4DMT is metabolized to the corresponding deutero-*N*-methyltryptamine, tryptamine, 1,2,3,4-tetrahydro-beta-carboline, and 2-methyl-1,2,3,4-tetrahydro-beta-carboline in rat brain. The subcellular distribution of D_4DMT and the aforementioned metabolites is also reported.

This laboratory has presented data concerning the metabolism of [5-^{3}H]-*N*,*N*-dimethyltryptamine (DMT) in rat brain homogenate [1]. In addition to the identification of the known *in vitro* and/or *in vivo* metabolites of DMT, i.e. indoleacetic acid (IAA), DMT-*N*-oxide (DMT-NO), tryptamine (TA) and *N*-methyltryptamine (NMT) [2–4], two previously unreported metabolites of DMT were identified: 1,2,3,4-tetrahydro-beta-carboline (THBC) and 2-methyl-THBC (2-MTHBC) [1]. The purpose of the present study was to examine the relevance of these observations to the metabolism of DMT *in vivo* and to further characterize the metabolic pathways involved. Thus, we report here the time course for the formation of some of these metabolites in rat brain following an intraperitoneal injection of α,α,β,β-tetradeutero-DMT (D_4DMT).

Gas chromatographic/mass spectrometric/selected ion monitoring/isotope dilution (GC/MS/SIM/ID) data are presented, quantifying the brain levels of D_4DMT and its corresponding deutero-metabolites (deutero TA, NMT, THBC and 2-MTHBC) as a function of time and dose. The subcellular distribution of D_4DMT and of the above deutero-metabolites in rat brain is also reported.

MATERIALS AND METHODS

Standards and reagents. Tryptamine·HCl (TA) and glyoxylic acid·H_2O were obtained from the Aldrich Chemical Co., Milwaukee, WI. A sample of α,α,β,β-tetradeutero-TA·HCl (D_4TA, > 98% isotopic purity) was obtained from Merck Sharpe and Dohme Isotopes, Montreal, Canada. Authentic samples of D_4DMT (99.7% isotopic purity), α,α,β,β-tetradeutero-NMT (D_4NMT, 99.7% isotopic purity) and NMT were provided by Professor Fred Benington and Dr. Richard Morin of this laboratory and were synthesized by the method of Speeter and Anthony [5] using either $LiAlD_4$ or $LiAlH_4$. Samples of THBC, 1,2-dihydro-3,3,4,4-tetradeutero-beta-carbo-line (TDBC, 99.8% isotopic purity), 2-MTHBC and 2-methyl-TDBC (2-MTDBC, 99.6% isotopic purity) were prepared via the reaction of TA, D_4TA, NMT and D_4NMT, respectively, with glyoxylic acid, according to the method of Ho and Walker [6]. Heptafluorobutyrylimidazole (HFBI) was obtained from the Pierce Chemical Co., Rockford, IL. Solvents were of spectro-grade and were obtained from Burdick & Jackson Laboratories, Muskegon, MI. All other chemicals were obtained from commercial sources and were of the highest available purity.

Gas chromatography/mass spectrometry of standards. Samples of DMT, D_4DMT, TA, D_4TA, NMT, D_4NMT, THBC, TDBC, 2-MTHBC and 2-MTDBC (10 μg of each) were converted to their corresponding heptafluorobutyryl (HFB) derivatives [1, 7–9] for GC/MS analysis. The GC/MS characteristics of these derivatives were determined using a Hewlett–Packard 5985A GC/MS equipped with a data analysis system. Gas chromatography was conducted on a Supelco 1.22 m by 2 mm internal diameter glass column containing 2% SP 2250 (Supelco) on 100–120 mesh Chromosorb-W-HP. A temperature program was used to obtain efficient separation: 170° initial temperature, isothermal for 1.5 min, whereupon the temperature was raised to 260° at a rate of 25°/min. The injection and transfer line temperatures were maintained at 270°. High purity helium was used as the carrier gas at a flow rate of 40 ml/min. The effluent from the column was led directly into the mass spectrometer, which was operated in the electron impact mode at an ionizing energy of 70 eV. Analyses conducted in the selected ion monitoring

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‡ To whom reprint requests should be addressed. Present address: UAB GC/MS Center, University of Alabama in Birmingham, Birmingham, AL 352 94.

(SIM) mode were accomplished with an electron multiplier setting of 3000 V.

A mass spectrum of each derivative was obtained and characterized with respect to the base peak (normalized to 100%) and other prominent secondary mass fragments for use in SIM analysis and determination of isotopic purity. The retention times were noted, and the diagnostic mass fragments were monitored in the SIM mode. Ion (m/z) ratios were calculated, and the percent interference between the ions chosen for the respective proteo and deutero compounds was evaluated.

D_4DMT loading. Adult male Long–Evans rats weighing between 300 and 350 g were individually housed and fed and watered *ad lib.* The rats were divided into three groups of twenty rats each with individual groups receiving by i.p. injection 2.5, 5.0 or 10.0 mg D_4DMT/kg dissolved in pH 6.0 isotonic saline (pH adjusted with 0.1 N HCl). Four rats from each group were then killed at 5, 10, 20, 40 and 80 min post-injection by decapitation. The brains were rapidly excised, frozen in liquid N_2, and stored at −76° until the extraction was performed.

Preparation of brain samples for GC/MS analysis. The preparation of rat brain extracts for GC/MS analysis was performed by modification of methods previously described by this laboratory [1, 7–9]. All glassware was acid (HNO_3) washed and silanized (Pro-Sil, PCR Research Chemicals, Gainesville, FL).

Each frozen whole rat brain was placed in a tared glass homogenizing tube containing 2.0 ml of glass-distilled H_2O, 0.5 ml of 70% $HClO_4$ and 1000 ng/g brain tissue of each of the proteo internal standards (DMT, TA, NMT, 2-MTHBC and THBC). The brain was thoroughly homogenized (Polytron PCV-2) and then centrifuged (International Clinical Centrifuge, setting of 5) for 30 min. The supernatant fraction was decanted, and the pellet was rehomogenized with an additional 2.0 ml of H_2O and 0.5 ml of 70% $HClO_4$. This was centrifuged for 25 min and the supernatant fractions were combined. The remainder of the extraction and derivatization procedure was conducted as previously described [8].

Subcellular fractionation of rat brain. Six rats were injected i.p. with 10.0 mg D_4DMT/kg and killed 40 min post-injection by decapitation. The brains were rapidly excised and subjected to the procedure described by Gray and Whittaker [10] for subcellular fractionation of brain tissue.

Individual brains were placed in 10.0 ml of ice-cold 0.32 M sucrose in glass homogenizing tubes and thoroughly homogenized with a Teflon pestle (0.025 cm clearance). The homogenate was centrifuged at 1000 g for 10 min (Sorvall, 4°), and the supernatant fraction was decanted (S_1). The pellet (P_1) was resuspended in 20.0 ml of 0.32 M sucrose and centrifuged at 1000 g for 10 min. This supernatant fraction (S_2) was combined with S_1 and the pellet (P_2) was saved. The combined supernatant fractions were centrifuged at 10,000 g for 20 min. The supernatant fraction (S_3) was decanted from the pellet (P_3) and lyophilized (Virtis Uni-Trap), and the residue was saved for GC/MS analysis. The P_3 pellet was resuspended in 0.32 M sucrose and layered on a gradient of 0.8 and 1.2 M sucrose (12.0 ml/layer). The samples were centrifuged at 20,000 g for 90 min (Beckman L-350 Ultracentrifuge, 4°) to give three fractions, A, B and C, between the layers. Fractions A and C were removed with pasteur pipettes and saved. Fraction B was transferred to a 15-ml conical tube and suspended in 5 vol. of Tris·HCl buffer (5.0 mM, pH 8.0) for 45 min to lyse the cells. The suspension was centrifuged (11,500 g for 20 min), and the supernatant fraction (S_4) was decanted. The pellet (P_4) was taken up in 5.0 ml of 0.32 M sucrose and stored at −76°. The S_4 supernatant fraction was centrifuged (100,000 g for 30 min), and the resulting

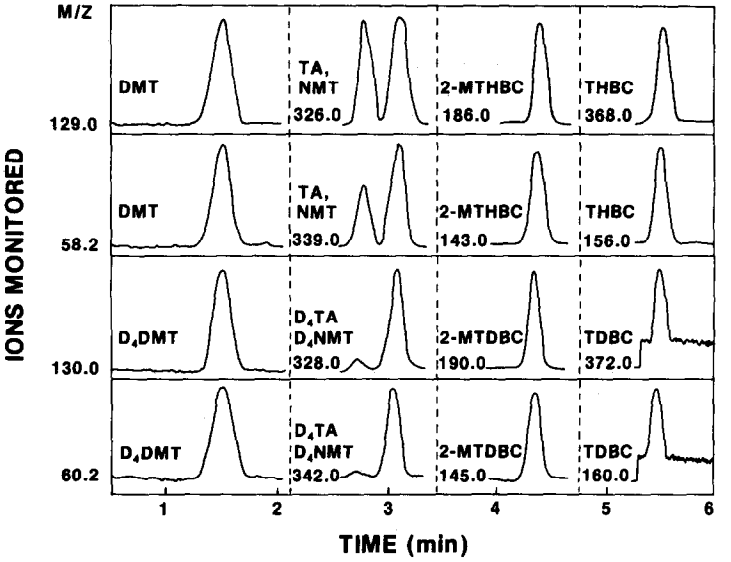

Fig. 1. Representative chromatogram of the GC/MS/SIM/ID analysis of rat whole brain, illustrating the retention times and ions (m/z) used for identification and quantification of the compounds examined. The sample shown was from the analysis of rat brain obtained 10 min post-injection of 10.0 mg D_4DMT/kg.

Table 1. Time- and dose-dependent concentrations of D_4DMT, D_4NMT, D_4TA, 2-MTDBC and TDBC in rat brain following i.p. injection of D_4DMT*

Dose (mg/kg)	Time (min)	D_4DMT	D_4NMT (ng/g)	D_4TA	2-MTDBC	TDBC
2.5	5	5,095.0 ± 672.0	20.1 ± 0.5	1.8 ± 0.1	0.0	0.0
	10	720.0 ± 184.2	4.6 ± 1.8	1.3 ± 0.2		
	20	785.0 ± 7.4	4.1 ± 2.6	2.2 ± 0.9		
	40	1,045.1 ± 21.0	18.3 ± 17.4	4.4 ± 3.3		
	80	37.9 ± 0.6	1.3 ± 0.8	1.4 ± 1.3		
5.0	5	4,140.0 ± 1,556.0	15.6 ± 5.2	3.4 ± 1.6	0.0	0.0
	10	2,297.0 ± 1.205.6	32.7 ± 12.9	2.9 ± 1.3	14.1 ± 2.6	0.6 (N = 2)
	20	1,807.0 ± 633.1	22.3 ± 0.1	1.3 ± 0.1	24.0 ± 6.8	0.5 (N = 2)
	40	1,880.2 ± 877.1	32.4 ± 3.0	2.2 ± 0.5	20.0 ± 2.4	0.0
	80	41.3 ± 7.4	0.7 ± 0.1	0.0	43.0 ± 8.8	0.0
10.0	5	1,490.1 ± 867.0	2.1 ± 1.5	2.0 (N = 2)	0.0	0.0
	10	8,464.0 ± 1,462.0	50.4 ± 10.7	3.4 ± 2.8	32.1 ± 4.0	2.7 (N = 2)
	20	14,489.0 ± 4,052.0	125.0 ± 18.5	5.0 ± 1.7	70.5 ± 18.5	0.5 (N = 2)
	40	8,178.0 ± 542.6	77.0 ± 23.0	4.8 ± 3.7	68.7 ± 20.0	0.8 (N = 2)
	80	1,045.0 ± 369.8	0.0	0.0	93.0 ± 17.0	0.0

* Values are expressed as means ± S.D.; except where noted otherwise, N = 4.

supernatant fraction (S_5) and pellet (P_5) were separated and stored at −76°.

Aliquots (0.1 ml) from suspensions of the P_2, A, C, P_4 and P_5 and from the S_3 and S_5 fractions were removed and stored at −5° for protein determination [11]. The remaining material was processed through the procedure described for the preparation of samples for GC/MS analysis [8] and were examined for the relative distribution (ng/mg protein) of D_4DMT and its metabolites in the fractions.

Limits of detection and linearity of recovery. Groups of six rats each were decapitated, and their brains were pooled in 12.0 ml of H_2O containing 3.0 ml of 70% $HClO_4$ and then thoroughly homogenized. The homogenate was divided into six equal fractions and 2000 ng of each of the proteo internal standards were added. Increasing amounts of the corresponding deutero compounds were then added (0, 2, 20, 200, 1000, 2000 ng of each), and the samples were vigorously mixed (Vortex). These samples were subjected to the procedure described above for extraction and GC/MS analysis and were used to determine the limit of sensitivity and the linearity of recovery for the assay.

RESULTS

The mass spectra of the HFB derivatives of the compounds examined here have been reported previously [8, 9, 12, 13]. A representative chromatogram, illustrating the GC/MS/SIM/ID analysis of rat whole brain tissue for the quantification of the HFB derivatives of D_4DMT, D_4NMT, D_4TA, TDBC and of 2-MTDBC using the corresponding proteo compounds as internal standards, is presented in Fig. 1. The results of the analyses are presented in Table 1 and show the time- and dose-dependent concentrations of these compounds in brain. The results obtained from the fractionation of brain tissue from rats 40 min post-injection of 10.0 mg D_4DMT/kg are shown in Table 2. The values reported have been corrected for ion interference between the proteo and deutero compounds, and identification was based on peak retention time, presence of diagnostic ions and ion intensity ratios, as determined from the analysis of standards.

The recoveries of D_4DMT, D_4TA, D_4NMT, TDBC and 2-MTDBC were > 90% and were linear over the range of concentrations examined, having

Table 2. Concentrations of D_4DMT, D_4TA, D_4NMT and 2-MTDBC in brain fractions 40 min post-injection of 10.0 mg D_4DMT/kg*

Fraction	D_4DMT	D_4TA (ng/mg protein)	D_4NMT	2-MTDBC
P_2	14.30 ± 3.06	0.248 ± 0.068	0.038 ± 0.007	0.650 ± 0.482
S_3	136.64 ± 16.79	0.513 ± 0.093	0.629 ± 0.097	1.437 ± 0.689
A (My)	12.51 ± 4.59	0.137 ± 0.015	0.095 ± 0.028	0.0
C (Mito)	1.69 ± 0.457	0.466 ± 0.309	0.015 ± 0.011	0.962 ± 0.378
P_4 (SPM)	0.396 ± 0.171	0.256 ± 0.059	0.023 ± 0.009	1.29 ± 0.893
S_4 (VS)	7.86 ± 1.75	0.967 ± 0.653	0.669 ± 0.633	0.217 ± 0.216
P_5 (V)	2.01 ± 0.767	1.20 ± 0.205	0.246 ± 0.089	18.04 ± 5.33

* Values are expressed as mean ± S.D., N = 6. Abbreviations: P_2 = particulate; S_3 = soluble; My = myelin; Mito = mitochondria; SPM = synaptic plasma membrane; VS = vesicle supernatant; and V = vesicles.

r values of 0.98, 0.99, 0.99, 0.99 and 0.98 respectively. The limits of detection for deuterated DMT, TA, NMT, THBC and 2-MTHBC were calculated to be > 1.0, 0.5, 1.0, 0.5 and 1.0 ng/g of brain tissue respectively.

DISCUSSION

Applicability of the method. As can be seen from Fig. 1, the GC method utilized here provides efficient separation of the compounds under examination. The excellent linearity of recovery and low ng/g limits of detection make this method suitable for the study of DMT in biological samples. We have shown previously that the extraction method employed here does not lead to the artifactual formation of any of the compounds in question [1, 8, 9], including the possible artifactual formation of the beta-carbolines from HCHO and the corresponding indolethylamine.

The kinetic isotope effect. The mechanisms for the formation of beta-carbolines and the demethylation and N-oxidation of DMT are not proposed to involve either the α- or β-positions of the ethylamine sidechain [1]. Thus, the substitution of D for H in these positions would be expected to exert a secondary and thus small kinetic isotope effect on these pathways [14]. However, we have observed recently that the brain levels of D_4DMT are 2.7 ± 1.1 times higher than those found following an i.p. injection of an equal dose of DMT [15]. This potentiation of brain level by the substitution of D for H is translated into a potentiation of the behavior disrupting effects of DMT as well [16]. The results of these aforementioned studies provide an indication that DMT may be significantly metabolized by direct deamination at the α-position, leading to the formation of IAA. Since the rate of deamination is somewhat slower for D_4DMT, due to a primary kinetic isotope effect [15], one would also predict that "metabolic switching" or "shunting" of D_4DMT into other metabolic pathways would occur [14]. Therefore, the levels of the metabolites observed in the present study are most likely higher than would be observed for DMT itself. This is a fortuitous result since it allows for the closer examination of these pathways without the use of MAO inhibitors, which may possess metabolic effects other than MAO inhibition alone [1].

Brain levels of D_4DMT. The brain levels of D_4DMT as a function of time and dose are presented in Table 1. The measurement of these levels serves as a method for comparing the relative amounts of each metabolite to the level of precursor present. As we have reported previously [15], D_4DMT shows rather different dynamics of uptake and clearance compared to DMT.

Formation of D_4NMT. The formation of D_4NMT closely paralleled the brain level of D_4DMT (Table 1). However, the total amount of D_4NMT present represents only 1.2 ± 0.89, 1.6 ± 0.5 and 0.63 ± 0.33% (mean for the five time points ± standard deviation) of the brain D_4DMT levels produced by injection of 2.5, 5.0 and 10.0 mg D_4DMT/kg, respectively. This may be indicative of the reported rapid metabolism of NMT by monoamine oxidase (MAO) and aldehyde dehydrogenase to IAA [17]. However, one would also predict that D_4NMT would not be as rapidly metabolized by MAO as NMT itself, due to a primary kinetic isotope effect produced from the substitution of D for H on the α- and β-positions. Thus, an alternative explanation for the relatively low levels of D_4NMT observed is that DMT is not significantly metabolized via demethylation in brain tissues and that NMT produced in the periphery is not significantly redistributed to the brain via the circulation. A complicating factor in this determination is the possibility that D_4NMT may also be remethylated to form D_4DMT or may serve as an immediate precursor for the formation of 2-MTDBC [1].

Formation of 2-MTDBC. Consistent with the results reported by this laboratory for the *in vitro* metabolism of DMT [1], 2-MTDBC was identified as a metabolite of D_4DMT in rat bain *in vivo* (Table 1). Although detectable amounts of 2-MTDBC were not observed at the 2.5 mg D_4DMT/kg level, the formation of 2-MTDBC from 5.0 and 10.0 mg D_4DMT/kg closely paralleled one another in a near dose-dependent fashion (Table 1). However, the levels of 2-MTDBC observed were not well correlated with those of D_4DMT. One possibility to be considered here is that the pattern observed for the formation and continuing accumulation of 2-MTDBC in brain may be due, in part, to its production in the periphery and its subsequent transport into brain tissues. This possibility is a matter for further inquiry. One might also speculate that the observed continued increase in 2-MTDBC is due to its possible storage in brain vesicles, thus being protected from degradation or clearance via blood circulation.

This laboratory has reported previously that approximately 50% of the 2-MTHBC formed during the metabolism of DMT *in vitro* is apparently produced via non-enzymatic mechanisms involving the condensation of NMT with formaldehyde derived from the demethylation of DMT and DMT-NO [1, 3, 18], during incubation. However, the mechanisms proposed for the demethylation of tertiary amines such as DMT are identical to those proposed in the Pictet–Spengler condensation of amines with formaldehyde: initial formation of a carbinolamine followed by dehydration to form an iminium ion [1]. Thus, 2-MTDBC may have been formed *in vivo* from a Pictet–Spengler condensation between D_4NMT and formaldehyde or from the condensation of the intermediate iminium ion formed during the demethylation of D_4DMT. The apparent low level of demethylation observed in brain supports the possibility that a large portion of the 2-MTDBC formed may have been derived from peripheral sources or that cyclization of the iminium ion intermediate effectively competes with demethylation processes.

Formation of D_4TA and TDBC. In further agreement with the results obtained for the metabolism of DMT *in vitro* [1], D_4TA and TDBC were observed to be formed as apparently trace metabolites of D_4DMT (Table 1). However, the formation of these metabolites was not observed to follow a dose–dependent function or to correlate in any significant way with the prevailing brain levels of D_4DMT. The

brain levels of D_4TA and TDBC approached the limits of detection for these compounds, and the results obtained for their quantification were, at times, highly variable. The formation of TA as an *in vivo* metabolite of DMT has been reported previously [3, 4] and apparently occurs via a didemethylation sequence. The TDBC may arise via a Pictet–Spengler condensation of D_4TA with formaldehyde or by mechanisms similar to those described for 2-MTDBC formation occurring during the further demethylation of D_4NMT. The possible demethylation of 2-MTHBC should also be considered. Nevertheless, as is evident from the results presented here, the formation of TA and THBC as metabolites of DMT in rat brain *in vivo* is an apparently minor pathway. However, these pathways may prove to be of more significance in peripheral tissues.

Subcellular distribution of D_4DMT and its metabolites in rat brain. The fractionation of rat brain was carried out according to the procedure of Gray and Whittaker [10] for the isolation of synaptic vesicles. However, since electron microscopic and tracer enzyme analyses were not conducted on these fractions, their actual cellular content cannot be stated with certainty. The results presented in Table 2 are thus preliminary in nature and descriptive in content.

As can be seen from Table 2, the D_4DMT in rat brain, derived from the administration of 10 mg D_4DMT/kg followed by sacrifice 40 min post-injection, is mainly associated with the S_3 or "soluble" fraction. Significantly smaller amounts were observed in the P_2 (crude particulate), A (myelin), C (mitochondria), P_4 (synaptic plasma membrane), S_4 (vesicular supernatant) and P_5 (vesicles plus microsomes) fractions.

The D_4NMT was observed to be mainly associated with the S_3, S_4 and P_5 fractions. However, the standard deviation for the S_4 fraction was quite high (Table 2).

The 2-MTDBC was observed to be associated mainly with the P_5 fraction. This may serve as an indication that 2-MTDBC is actively transported into synaptosomes and stored in synaptosomal vesicles. The elucidation of this possibility is a matter for further research. Detectable amounts of TDBC were not found in any of the fractions examined, possibly due to a wide distribution of this trace metabolite. However, TA was also observed to be most predominantly associated with the P_5 fraction.

Interpretation of the distribution of D_4DMT in these fractions must be viewed with caution since significant contamination of fractions may have occurred during the homogenization process. Thus, the question of whether or not these compounds are actively transported into synaptosomes and sequestered in vesicles cannot be sufficiently addressed. However, endogenous DMT has been shown to be located in the corresponding S_4 and P_5 fractions of rat brain [19]. While the distribution of D_4NMT appears to be somewhat diffuse, the apparent concentration of 2-MTDBC into the P_5 fraction is a most interesting observation.

Relationship to future studies of DMT in vivo. It is of interest to note that the highest enzyme activities for the biosynthesis of DMT reside in tissues other than the brain [18]. It has been clearly demonstrated that DMT administered via the periphery is rapidly and, perhaps, actively transported into brain tissue [18]. Thus, the biosynthesis and metabolism of DMT in brain tissues are not the sole factors to be considered when one examines the possible role of DMT *in vivo*. Furthermore, the apparent rapid metabolism of DMT *in vivo* does not allow for one to consider a measurement of DMT alone to be an accurate reflection of prevailing DMT levels. Such studies fail to consider other factors which would be indicative of the overall turnover rate of this hallucinogen. Future studies should focus on the measurement of key precursors, intermediates and metabolites of DMT as well. This is especially the case in studies which have attempted to correlate body fluid levels of DMT with a diagnosis of schizophrenia.

We have shown that D_4DMT is metabolized to D_4NMT, 2-MTDBC and trace amounts of D_4TA and TDBC in rat brain *in vivo*. The use of this deuterated analog of DMT has allowed for the examination of these pathways without the use of pharmacological intervention, i.e. MAO inhibitors. The data appear to indicate that D_4DMT, at the levels examined here, is not significantly metabolized in brain but is rather rapidly transported into and cleared from brain tissues via blood circulation. Thus, the most extensive metabolism of DMT probably occurs in peripheral tissues, especially in liver [2]. It is further proposed that 2-MTHBC may be formed, in part, in the periphery and transported into brain. This is a matter for further inquiry. However, the data obtained from the analysis of the subcellular distribution of D_4DMT and its metabolites seem to indicate that 2-MTDBC and D_4DMT may be concentrated into synaptosomes and synaptosomal vesicles. This is of interest since 2-MTHBC has been identified as a trace constituent of rodent brain [9].

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